Remarks

Cancellation and Amendment of Claims

In light of the Examiner's remarks and rejections, the Applicants' Agent has herein amended Claim 64 and added new Claims 101 – 103. Neither the amendment of Claim 64 nor the new Claims 101 – 103 contain new matter.

All claims are readable on the species elected on August 2, 2004, i.e., the vector encoding the T7B peptide extension identified as SEQ ID NO: 6.

The Applicants respectfully request the Examiner's review of the amended Claim and the newly added claims, particularly in view of the following remarks.

1. Rejection under 35 USC 112, first paragraph: Written Description:

Claim 64 had been rejected as noted. The Applicant's Agent acknowledges the withdrawal of the rejection as to Claim 64.

2. Rejection under 35 USC 112, second paragraph: Indefiniteness:

In response to the rejection of claim 64 under 35 USC 112, second paragraph, Indefiniteness, the Agent for the Applicants has, herein, amended Claim 64 in an effort to address the rejection remarks of the Examiner. As follows, the Agent for the Applicants respectfully submits that the claim is now in condition for allowance.

The Examiner noted that Claim 64 contained "poor solubility", which was considered to be a relative term causing the claim to be indefinite. The Agent for the Applicants has, therefore, removed the term from the claim in the present amendment. Because the preamble of the claim indicates that the vector(s) of the claimed invention are

for enhancing solubility of the encoded proteins and polypeptides of interest, one of skill in the art deciding to make use of the claimed vectors would either (1) know that the encoded protein or polypeptide of interest was poorly soluble if expressed in other vectors and thus expression in the claimed vectors would be advantageous or (2) would think that expressing the protein or polypeptide of interest as a fusion with the claimed peptide extensions would be desirable (or at the very least, acceptable) even in advance of knowing whether the protein was or was not poorly soluble when expressed in the bacterial host cell without the peptide extension.

Lastly, although the Agent has deleted the purportedly indefinite term from the amended Claim 64, it must be noted that those practicing this art and those having ordinary skill, creativity and experience in the art know the meaning of "poor solubility". To such individuals it is commonly associated with either a poor yield (i.e., the poorly soluble, generally misfolded proteins are degraded by host proteinases) or the formation of inclusion bodies within the host cells. This is pointed out in the specification – the Examiner is directed to line 19, page 2 through line 5, page 3 of the specification as filed. This is further supported in the specification, for example, see line 20, page 5 through line 15, page 6 wherein the "virtues" of expressing proteins or polypeptides of interest as fusions to enhance their solubility is summarized. Lastly, it is generally accepted that "poor solubility" results in large part from the inability of the over-expressed protein or polypeptide of interest to achieve "proper folding" in the over-expression environment. The actual causes for such "poor solubility" and inability to achieve "proper folding" are

poorly understood, but as mentioned above, result in poor yields or the formation of insoluble (the extreme form of "poor solubility") inclusion bodies within the host cell.

Thus the Agent submits that the amended Claim 64 is now in condition for allowance.

3. Rejection under 35 USC 112, first paragraph: Written Description – New Matter:

The Examiner cites "poor solubility" in the context of claim 64 to be new matter and has additionally rejected Claim 64 under 35 USA 112, first paragraph.

The Agent for the Applicants has, herein, amended Claim 64 to eliminate the terminology. Therefore the Agent submits that this rejection is therefore rendered moot.

In addition, the Agent respectfully draws the Examiner's attention to the "Synopsis of Application of Written Description Guidelines" – ". . . the examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claim. Clear evidence or reasons to interpret "poor solubility" as new matter do not appear to be present in the Office Action, particularly in light of the above citations from the specifications as well as the level of skill typical in the art and the level of understanding of the meaning of the term "poor solubility".

4. Rejection under 35 USC 103(a).

Joint inventors: The named inventors contributed to each of the claims remaining in this application. Their inventive contributions were made prior to the initial date of filing of this invention, and can be traced to preceding the date of preparation of the initial draft

of the application, and preceding the date of signing of the Record of Invention filed with our office at Brookhaven, i.e., prior to November 7, 2000.

Obviousness: Claim 64, which reads on:

> "any pET-15b expression vector comprising a first nucleic acid sequence encoding a peptide extension, wherein the encoded peptide extension is Peptide T7B (SEQ ID NO:6), the expression vector further comprising a multiple cloning site",

was rejected as unpatentable over Freimuth et al. (J. Virol. (1991) **73**:1392-1398) in view of Condron et al. (J. Bacteriol. (1991) **173**:6998-7003).

On page 6 of the Office Action, the Examiner states that:

> "Freimuth et al differ from the invention claimed in the instant Claim 64 because Freimuth et al fail to teach the peptide extension **is the** T710B (SEQ ID NO:6). It is noted that SEQ ID NO:6 is **a 44 amino acid sequence** that begins with the first four amino acids of the peptide extension of Freimuth et al (LEDP) contiguously in sequence with the carboxyl-terminal 40 residues of the phage T7 gene 10B protein." (emphasis added)

In the cited Freimuth et al article, a fortuitous cloning of the extracellular domains of the **c**oxsackie virus/**a**denovirus **r**eceptor (CAR) protein, including the D1 and the combined D1/D2 domain, into pET-15b using the *Nco*I-*Xho*I restriction sites lead to the D1 domain (as well as the D1/D2 domain) being expressed as a fusion protein in which the vector-encoded peptide sequence LEDPAANKARKEAELAAATAEQ (peptide T7A, SEQ ID NO: 20 of the present application) was fused at the carboxyl terminus of the D1 domain. The resultant expressed D1 fusion protein was found to be more soluble (but only at 18°C) than the D1 domain expressed without the 22 amino acid extension (i.e., when cloned in pET20b or when cloned in pET 15b with a stop codon inserted in such a way as to eliminate fusion with the 22 amino acid extension) (see, in Freimuth et al the paragraphs of

the section entitled "Expression and purification of CAR extracellular fragments", pages 1393 – 1394).

Condron et al teach that the product of gene 10 of bacteriophage T7 (i.e., the phage gene 10 protein) is expressed in two forms, the normal, 10A form and an extended, 10B form as a result of a frameshift occurring during translation. The 10B protein contains 52 extra amino acids at the carboxyl terminus compared to the 10A protein. Condron et al, and particularly Dunn and Studier (J. Mol. Biol. (1983) **166**:477-535), teach that the 10B protein is formed as a result of a frameshift during translation and the aberrant 10B protein then becomes a minor component of the T7 phage capsid. The amino acid sequence comprising the 52 amino acid extension (Condron et al) was originally implied from the known sequence of the phage T7 DNA. The -1 frameshift leading to the 10B protein was first taught in Dunn and Studier, J. Mol. Biol. (1983).

The Agent for the Applicants respectfully submits that Freimuth et al could not have taught (and therefore "fail to teach") "the peptide extension is the T710B (SEQ ID NO:6) (*sic*)". The peptide extension of Freimuth et al **is not** the T710B peptide (SEQ ID NO: 6) and is not a 44 amino acid sequence but **is** T7A, a 22 amino acid sequence, noted SEQ ID NO:20 in the present specification. The Agent further points out that the referenced SEQ ID NO:6) is named peptide extension **T7B and not T710B**. Is it possible that there is some confusion in an unfortunate overlap in nomenclature between the phage 10B protein and the peptide extension designated as T7B (SEQ ID NO:6) and perhaps the peptide extension T7B10 (SEQ ID NO:16)?

Continuing on pages 6 through 7, the Examiner states:

"The Score Report, in light of Condron et al teach the amino acid sequence of the T7 gene 10B protein, including the carboxyl-terminal 40 residues."

"Absent evidence to the contrary, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have substituted the 40 amino acid residue sequence for the 18 amino acid residue sequence of the phage T7 gene 10B protein (see Score Report of SEQ ID NO:6 and Cordon (*sic*) et al) in the pET 15b vector containing the 22 peptide extension taught in Freimuth et al because **Cordon** (*sic*) (see Score Report) **teach the 40 amino acid sequence of the carboxyl-terminal 10B protein was known and Freimuth et al proposed using mutagenesis approaches, regarding the 22 amino acid peptide extension study, for optimization of Ad2 knob solubility.** . . . Since the 22 amino acid extension consisted of the last 18 amino acids of the 10B gene, it would have been obvious to one of ordinary skill in the art for the "mutagenesis" regarding the solubility peptide extension to include addition of the next contiguous amino acids starting from the last 18 amino acids of the 10B gene, as shown in Applicants invention (page 1397, right column, paragraph 2).

First, while agreeing that Condron et al (and particularly through work of Dunn and Studier, J. Mol. Biol. (1983)) teach the extension of the phage 10A protein to form the 10B protein, the Agent rebuts the obviousness of the choice of the carboxyl terminal 40 amino acids of the phage T7 10B protein to form peptide extension T7B (SEQ ID NO:6). If this had been an obvious choice, why wasn't it obvious to choose the last 35 amino acids, or the last 28 or the last 48? The list of choices as to what to "substitute for the 18 residue sequence" is extensive – and could have been peptides increasing monotonically in length with each additional amino acid starting with the 18 of Freimuth et al. and extending to the complete phage T7 10B protein 52 amino acid extension (i.e., to the claimed T7C peptide extension (SEQ ID NO:5)). Instead the invention claimed (in addition to peptide T7C) is the 40 amino acid sequence and amino acid variants thereof as well as amino acid variants of the original 18 amino acids taught by Freimuth et al.

With respect to the remarks suggesting that Freimuth et al proposed using "mutagenesis approaches, regarding the 22 amino acid peptide extension study, for optimization of Ad2 knob solubility", the Agent for the Applicants respectfully submits that the Examiner has misrepresented the proposed uses of mutagenesis in the teachings of Freimuth et al. (As an aside, the Agent further notes that the peptide extension was not used in conjunction with expression of any of the adenovirus knob proteins in Freimuth et al or in the work cited in Freimuth et al.) Freimuth et al propose using mutagenesis for two very different purposes than the one suggested in the Examiner's remarks.

In Freimuth et al, the word mutagenesis appears twice. First, in the left column of page 1397, wherein Freimuth et al suggest using mutagenesis approaches to examine which amino acids of the CAR protein and of the adenovirus knob protein are important in generating the specific interaction between these two protein entities: "The importance of these amino acids to knob-CAR binding could readily be tested through site-directed mutagenesis of the recombinant proteins we describe here." This most assuredly has nothing to do with modifying the peptide extension of Freimuth et al to form the peptide extensions of the invention under examination.

The second recitation of "mutagenesis" in Freimuth et al appears in the penultimate paragraph of the article in the right column of page 1397: "Optimization of Ad2 knob solubility through mutagenesis approaches could provide insights into intrinsic factors that regulate protein folding and multimerization in vivo." This follows a discussion of the differences in the yields and solubility of the recombinant knob proteins from two different adenovirus serovars, Ad12 and Ad5 using bacterial expression systems. This discussion

concludes: ". . . therefore, the differences in yields of the soluble knob proteins must reflect differences in knob folding and trimerization in bacterial cells." This suggested use of mutagenesis has nothing at all to due with amino acid substitutions of the peptide extension of Freimuth et al nor the variants presently claimed.

The Agent submits that neither of the proposed uses of mutagenesis relate at all to substituting the 18 amino acid extension with a longer extension. Furthermore, and perhaps more importantly, neither of the proposed uses of mutagenesis in Freimuth et al would include changing the T7 phage gene 10B sequence (as is done in the present invention) to create peptide extensions having altered amino acids that further enhance the solubility-enhancing properties of the peptide extensions and which variant peptide extensions are claimed in the present invention.

Continuing on pages 7 through 8, the Examiner states:

> "One would have been motivated at the time the invention was made to have extended the peptide extension substituting the longer peptide extension containing the carboxyl-terminal 40 amino acid residues of the page T7 gene 10B protein because Freimuth et al stated that the solubility of a D1 protein **depended on the presence of the *22* amino acid peptide extension (page 1397, left column, paragraph 4)** but that the solubility of a different protein of interest, the Ad2 protein, was not as effected (*sic*) by the same vector sequences and further stated that "optimization of Ad2 knob solubility through mutagenesis approaches could provide insights into intrinsic factors that regulate protein folding and multimerization in vivo (page 1397, right column, paragraph 2)."

The Agent first respectfully points out the above discussion on the proposed mutagenesis studies of Freimuth et al. Second, please note the parenthetic remark on page 11 of this paper. None of the adenovirus knob proteins discussed in Freimuth et al. were expressed with a peptide extension. Thus, it would have been impossible to determine

whether or not, as the Examiner states, "the Ad2 protein, was not as effected by the same vector sequences".

Third, the Agent wishes to point out the neither Condron et al nor the work of Dunn and Studier (1983) suggest that the T7 10B protein is or is not more soluble than the shorter, normal T7 10A protein. If they had made some suggestion of that nature one may have been motivated to substitute the entire gene 10B extension for the 22 amino acid extension. However this teaching as motivation is absent.

In addition although Freimuth et al state that the solubility of a D1 protein depended upon the presence of the 22 amino acid extension on page 1397, on page 1394, right column, at the end of the first partial paragraph they also state, perhaps more accurately, that ". . . these data indicate that D1 solubility in *E. coli* cells is **enhanced** by the pET15b-encoded 22-amino acid carboxy-terminal extension". This is because D1 was only soluble when expressed as a fusion if expression was carried out at 18°C. This is further supported in the specification – see page 29, lines 1 through 6.

In addition, it is unclear that one of skill in the art would be motivated to substitute a longer peptide extension merely because the 22 amino acid peptide extension enhanced the solubility of the D1 domain of the CAR protein. There is little motivation supplied in the cited references either singly or in combination. This is particularly evidenced in the present specification in which it was clearly demonstrated that 22 amino acid peptide extensions having amino acid substitutions of the 22 amino acid peptide extension of Freimuth et al served to better enhance the solubility of D1 without resorting to the use of a longer sequence (see lines 1 through 7, page 34 of the specification).

Continuing on pages 7 through 8, the Examiner states:

> "Absent evidence to the contrary, one would have a reasonable expectation of success combining the teachings of the art because Freimuth et al showed that the substitution of **various length peptide extensions** into expression vectors was successfully practiced at the time Freimuth et al and Cordon (*sic*) et al were published."
>
> "In view of the foregoing, the vector of claim 64, as a whole, would have been obvious to one of ordinary skill in the art at the time the invention was made. Therefore, the claims are properly rejected under 35 USC *§103(a)*."

In response, the Agent for the Applicants respectfully rebuts the suggestion of a reasonable expectation of success, particularly as reasoned in the statement of the Examiner. The Agent offers the above remarks and the following analysis and reasoning in a bona fide attempt to move this case to allowance.

The Agent and the Applicants fully agree that Condron et al teach (especially in combination with Dunn and Studier (1983)) the carboxyl-terminal 40 residues of the T7 gene 10B protein. However, the Agent for the Applicants respectfully submits that the fortuitous cloning and resultant enhancement of solubility of the CAR D1 protein through extension with the 22 amino acids in Freimuth et al would NOT obviously or otherwise lead one to predict or assume one would achieve success by substituting a longer peptide extension. This is further supported by noting that Condron et al and Dunn and Studier make no suggestions or teachings that the phage T7 10B protein is or is not "more soluble" than the 10A protein. Thus, the revelation of the sequence of the T7 gene 10B as an extension of the T7 gene 10A protein of T7 phage capsids in Condron et al and the 22 amino acid extension of Freimuth et al would not lead one to suggest or imply or expect success with a 44 (40 amino acids from gene 10B protein plus LEDP) amino acid

extension. Again, as above, if merely lengthening the extension was better, why not extend the 22 amino acid peptide to 35 amino acids, or 28 or 48?

Further, if it was a general phenomenon that the "gene 10B type" extensions were appropriate for enhancing solubility and proper folding of recombinant proteins, one might expect that the phage T3 10B extension would be appropriate as well. As one can see in the present specification, the T3 gene 10B extension did not enhance solubility (page 28, line 10 through page 29, line 6).

The Examiner's comment: "Freimuth et al showed that the substitution of **various length peptide extensions** into expression vectors was successfully practiced at the time Freimuth et al and Cordon (*sic*) et al were published", is confusing such that the Agent for the Applicants cannot determine whether the Examiner is referring to the present specification showing that various length peptide extensions was successfully practiced or whether Freimuth et al showed use of various length peptide extensions. It is clear that the only "various length peptide extensions" that are taught are those of the present invention and that they were not taught in Freimuth et al. Because of this confusion and in light of the above rebuttal of the obviousness rejection, the Agent requests the Examiner's consideration of the above remarks as being persuasive that the invention being examined could not have been obvious under the combination of the cited references.

New Claims 101 – 103:

New Claims 101 – 103 have been added herein to claim bacterial expression vectors encoding the various peptide extensions, which vectors can be used to incorporate a nucleic acid sequence encoding a protein or polypeptide of interest such that following expression a

fusion protein composed of the protein or polypeptide of interest having a carboxyl terminal peptide extension is generated. These claims contain no new matter.

Summary

Claims remaining under consideration include currently amended Claim 64 and new claims101, 102 and 103 all readable upon the elected species – the vector encoding the T7B peptide extension (SEQ ID NO: 6) . The amendment and new claims contain no new matter.

In light of the above Amendments and Remarks, applicants respectfully submit that the instant application is now in condition for allowance and solicit a timely notice of allowance.

Respectfully submitted,

Christine L. Brakel
Agent for Applicants
Registration No. 45,772

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Christine L. Brakel
Patent Agent
Brookhaven National Laboratory
Bldg. 185
P.O. Box 5000
Upton, New York 11973-5000
(631) 344-7134